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Short Communication

OXA-48-Producing *Enterobacteriaceae* Causing Bacteremia, United Arab Emirates

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SUMMARY

OXA-48-producing isolates were identified in approximately 4% and less than 1% of ESBL-producing and non-ESBL-producing *E. coli* and *K. pneumoniae* causing bacteremia at the largest tertiary hospital in Abu Dhabi.

Carbapenems offer an important treatment option for infections caused by *Enterobacteriaceae*, in particular those producing extended-spectrum β-lactamase (ESBL). However, several classes of carbapenemases have emerged with the increased use of carbapenems. In the United Arab Emirates (UAE), the antimicrobial resistance rates of *Escherichia coli* and *Klebsiella pneumoniae* increased significantly between 1994 and 2006.[1] In terms of carbapenem resistance, detection of *Enterobacteriaceae* isolates producing NDM, and less frequently OXA-48, have been reported in the UAE.[2–4]

In the present study, we sought to systematically identify carbapenemase-producing *E. coli* and *K. pneumoniae* causing bacteremia at the largest tertiary medical center in Abu Dhabi, UAE. *E. coli* and *K. pneumoniae* clinical isolates identified from blood cultures were collected between 2011 and 2012. Only one isolate was collected per patient. A total of 80 ESBL-producing and 150 non-ESBL-producing isolates were collected, as determined by Vitek2 (bioMérieux, Marcy l'Etoile, France). The isolates were then screened for carbapenem non-susceptibility by growth on Luria-Bertani agar plates containing 0.5 μg/ml of ertapenem. As a result, 4 isolates (1 ESBL-producing *E. coli*, 2 ESBL-producing *K. pneumoniae* and 1 non-ESBL-producing *K. pneumoniae*) were confirmed to have reduced susceptibility to ertapenem and studied further. The 4 isolates were tested for the presence of bla_{SHV}, bla_{CTX-M}, bla_{KPC}, bla_{NDM} and bla_{OXA-48} genes by PCR amplification followed by confirmation with DNA sequencing. Minimum inhibitory concentrations (MICs) of various antimicrobial agents were determined by the broth microdilution method (Sensititre GNX2F, Thermo Scientific, Cleveland, OH) and interpreted according to the Clinical Laboratory Standards (CLSI) breakpoints. MICs of meropenem were determined with Etest (bioMérieux). Multilocus sequence typing (MLST) was performed according to published protocols (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html and http://mlst.warwick.ac.uk/mlst). De-identified medical records of the bacteremia cases were reviewed for demographic and clinical data. The study was approved by the Institutional Review Board of Sheikh Khalifa Medical City, Abu Dhabi.

All 4 patients with ertapenem-non-susceptible *Enterobacteriaceae* had healthcare-associated bacteremia (1 with *E. coli* and 3 with *K. pneumoniae*) (Table 1). Two were in an intensive care unit and on mechanical ventilation at the time of bacteremia. The APACHE II scores were high, ranging between 15 and 29. All patients were Arabic natives, but two of them had traveled overseas within 3 months prior to bacteremia (Saudi Arabia and the United Kingdom). All patients were treated with meropenem

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Table 1
Bacteremia cases due to OXA-48-producing *Enterobacterieaceae* in Abu Dhabi, UAE.

Case (Isolate)	Age	Sex	Ethnicity	Travel history	Admitting diagnosis	Underlying conditions	Therapy given	Meropenem MIC (μg/ml)	Survival at 14 days	ST	Concomitant ESBLs
1 (*E. coli* 12)	60s	Male	Arabic	Not recorded	Sepsis	Cirrhosis, diabetes	CTX then MEM	0.38	Deceased	4144	CTX-M-14, SHV-12
2 (*K. pneumoniae* 70)	70s	Female	Arabic	Saudi Arabia	Spinal fracture	Cardiovascular disease, diabetes	MEM	0.25	Survived	340	CTX-M-15
3 (*K. pneumoniae* c80)	50s	Female	Arabic	United Kingdom	Malignancy	Metastatic gastric cancer	TZP then MEM + CST	0.5	Survived	1425	SHV-12
4 (*K. pneumoniae* c82)	≤ 20	Female	Arabic	None	Malignancy	Acute lymphoblatic leukemia, neutropenia	MEM	0.38	Survived	29	None

MIC, minimum inhibitory concentration; CTX, cefotaxime; MEM, meropenem; TZP, piperacillin-tazobactam; CST, colistin.

for definitive therapy, and one received colistin in addition. The three patients with *K. pneumoniae* cleared bacteremia and were alive at 14 days after the positive culture, but the patient with *E. coli* bacteremia died from sepsis.

PCR and sequencing showed that all 4 isolates possessed the $bla_{\text{OXA-48}}$ gene whereas none had the $bla_{\text{NDM-1}}$ gene. The MICs of β-lactams reflected the presence or absence of ESBLs in addition to the ertapenem non-susceptibility due to production of OXA-48 (ertapenem MICs of ≥1 μg/ml), as non-ESBL-producing *K. pneumoniae* c82 was susceptible to cefotaxime but the other three isolates producing ESBL were resistant to it. The MICs of meropenem, which all patients received for therapy, ranged between 0.25 and 0.5 μg/ml. The low meropenem MICs were consistent with the favorable survival rate (3 of the 4 patients), though the number of patients was too small to make a definitive conclusion. All isolates remained susceptible to tigecycline and colistin.

By MLST, the isolates belonged to distinct clonal lineages. The *E. coli* isolate had a novel allelic profile ST4144, which represented a single locus variant (SLV) of ST38 which is commonly associated with OXA-48 production in Europe and the Middle East including Egypt and Lebanon.[5] The *K. pneumoniae* isolates belonged to ST340, 1425 and 29. ST340 is a SLV of ST258 and frequently associated with production of various carbapenemases including KPC.[6–8] ST29 had previously been reported for an OXA-48-producing isolate from Kuwait,[5] whereas ST1425 was a novel allelic profile.

We then screened the genetic environment of $bla_{\text{OXA-48}}$ by PCR mapping using *E. coli* TOP10 transformants harboring the $bla_{\text{OXA-48}}$-carrying plasmids and custom primers based on published sequences.[9] A copy of IS*1999* was present upstream of $bla_{\text{OXA-48}}$ in all 4 isolates. However, IS*1999* was intact in two isolates and interrupted by IS*1R* in the other two isolates. *lysR* was identified downstream of $bla_{\text{OXA-48}}$, without interruption by an IS element. These findings were overall in agreement with a previous study reporting the genetic support of $bla_{\text{OXA-48}}$.[9]

In summary, our study estimated the prevalence of $bla_{\text{OXA-48}}$ at approximately 4% and less than 1% among ESBL-producing and non-ESBL-producing *E. coli* and *K. pneumoniae* causing bacteremia, respectively, at the largest tertiary hospital in Abu Dhabi. However, the polyclonality of the isolates observed here is concerning for ongoing horizontal and regional spread of $bla_{\text{OXA-48}}$.

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Conflict of interest statement: Y.D. has served on an advisory board for Shionogi Inc., consulted for Melinta Therapeutics, and received research funding from Merck & Co. for a study unrelated to this work. All other authors: No potential conflicts of interest.

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